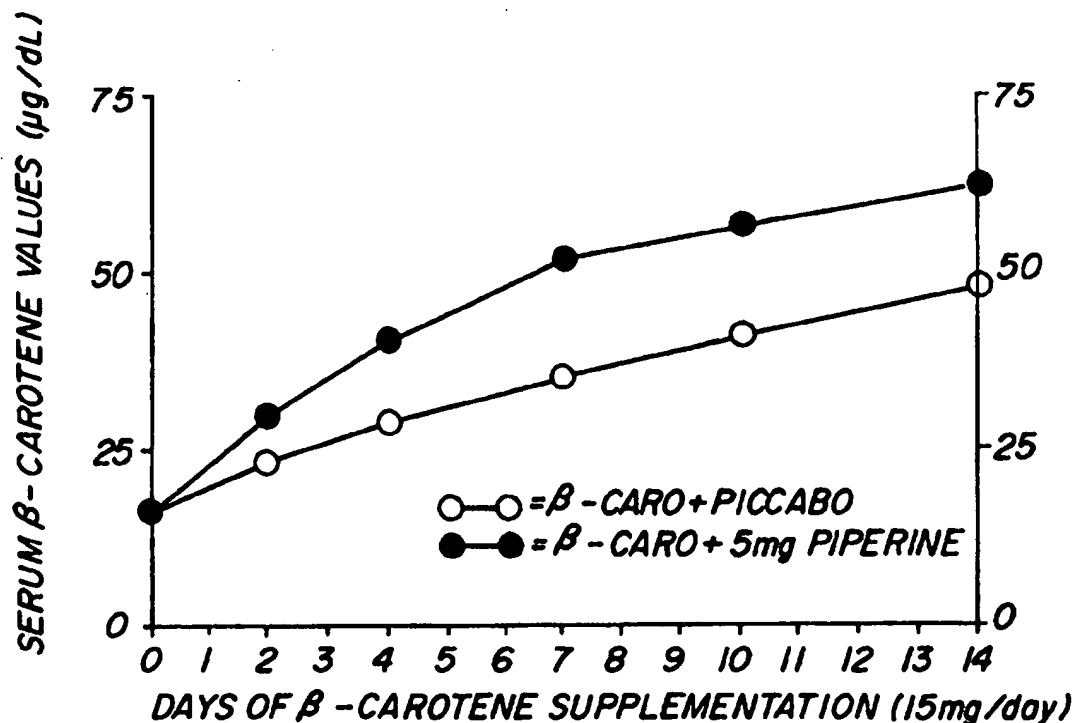


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(54) Title: USE OF PIPERINE AS A BIOAVAILABILITY ENHANCER



(57) Abstract

Compositions and methods for the improvement of gastrointestinal absorption and systemic utilization of nutrients and nutritional supplements, wherein the compositions comprise a minimum of 98 % of pure alkaloid piperine. The method comprises oral, topical, or parenteral administration of the compositions of the invention. A new process for the extraction and purification of piperine is also disclosed.

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USE OF PIPERINE AS A BIOAVAILABILITY ENHANCER

Background of the Invention

1. Field of the Invention

The present invention relates to a method for enhancing the bioavailability of nutritional compounds. The present invention also relates to compositions for such enhancement.

The fifty percent increase in life expectancy of Americans from 1930 to 1980 can, in part, be attributed to the improvement in nutrition in the United States during that period. However, the situation today remains far from ideal, since six out of ten of the leading causes of death in this country, including heart attack, cancer, cirrhosis of the liver, and diabetes, are linked to diet. It becomes increasingly obvious that many of those diseases could be prevented with a well balanced diet and efficient nutritional supplementation with certain vitamins and minerals.

The problem is particularly severe in older Americans. Approximately 30 percent of older Americans do not get the dietary requirements of all the essential nutrients. The hazards of food-drug interactions in depleting essential nutrients are well recognized. It is unavoidable that old age calls for increased use of medications. For example, use of certain antibiotics decreases absorption of calcium and iron, while EDTA chelation therapy decreases absorption of zinc, iron, copper, and magnesium.

In addition, many foods which increase the risk of cancer and cardiovascular disease have to be eliminated from the diet, which further depletes the sources of essential nutrients. For example, excellent sources of vitamin B and vitamin D, such as red meat,

liver, egg yolk, cheese and dairy products, are often limited because of their high cholesterol content.

Limited menu also causes a depletion of essential amino acids, such as tryptophan, which is important precursor of neurotransmitters, and may play a role in the prevention of brain deterioration with aging.

The availability of essential nutrients is further compromised by poor gastrointestinal absorption.

The traditional way to offset insufficient nutrient supplementation, insufficient gastrointestinal absorption and insufficient metabolic utilization of essential nutrients is to administer large doses of compensating materials, such as vitamin and mineral supplements.

The present invention provides an alternative method for improving nutritional status by increasing the bioavailability of various nutritional materials.

The bioavailability of nutrients is also relevant to animal health as well as human health. Thus, the compositions and methods of the invention are also intended to be used in veterinary practice.

2. Description of Related Art

Documents describing ayurvedic medicine dating from the period between the seventh century B.C. and the sixth century A.D. describe "trikatu". Trikatu is a Sanskrit word meaning three acids, and refers to a combination of black pepper (*Piper nigrum* Linn.), long pepper (*Piper longum* Linn.) and ginger (*Zingiber officinale* Rosc.). In traditional ayurvedic medicine these drugs are essential ingredients of many prescriptions and formulations used for a wide range of diseases. Experimental evidence shows that the use of "trikatu", and its constituents individually as well as

coll ctively, enhances the bioavailability of a number of drugs. In those studies carried out in animals as well as human volunteers, it was noted that the active component responsible for the increase in bioavailability of various drugs was piperine.

Piperine, or mixtures containing piperine, have been shown to increase the bioavailability, blood levels and efficacy of a number of drugs including ingredients of vasaka leaves (Bose, K.G., (1928) Pharmacopeia India, Bose Laboratories, Calcutta), vasicine (Atal et al., Journal of Ethnopharmacology, 4, 229-233 (1981)), sparteine (Atal et al., *ibid*), sulfadiazine (Atal et al., *ibid*), rifampicin (Zutshi, U. et al. (1984) Journal of the Association of Physicians of India, 33, 223-224), phenytoin (Bano et al., *Planta Medica*, 1987, pp. 568-569), pentobarbitone (Majumdar, A.N. et al. (1990), Indian Journal of Experimental Biology, 28, 486-487), theophylline (Bano et al., Eur. J. Clin. Pharmacol. (1991) 41:615-617) and propranolol (*ibid*).

The effect of piperine on the bioavailability of propranolol has been studied. The chronic oral administration of the anti-hypertensive agent propranolol is frequently rendered difficult due to the fact that steady therapeutic levels of this drug are not achieved or maintained. In addition, large doses are needed to be administered for efficacy and this frequently causes side-effects. Piperine has been shown to enhance the bioavailability of this drug.

Propranolol administered with piperine shows a significant increase in plasma levels of the drug, presumably due to decrease in metabolism by the liver.

Similar results have been obtained with piperine and vasicine, theophylline, and phenytoin. Piperine has also been added in multi-drug formulations

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for the treatment of tuberculosis and leprosy. A formulation containing rifampicin, pyrazinamide and isoniazid has been tested in human volunteers (Indian Patent No. 1232/DEL/89). For most drugs, the comparative levels and peak concentration of the drugs in the presence of piperine were higher. The applicability of these results to the development of anti-tuberculosis and anti-leprosy formulations, which are presently cost prohibitive in developing countries, is apparent. Bioavailability enhancement helps to lower dosage levels and shorten the treatment course.

In summary, all of these examples clearly illustrate the role of piperine as a drug bioavailability enhancer. The combination of piperine with tested drugs is effective primarily due to higher plasma concentration and a longer stay of the drugs in the body. The reduced dose of highly toxic drugs and their enhanced efficacy is obviously desirable.

The effective bioenhancing dose of piperine for drug compounds varies, but the prior art studies indicate that a dose of approximately 10% (wt/wt) of the active drug could be regarded as an appropriate bioenhancing dose for most drugs.

There are two plausible explanations of the role that piperine may have in drug bioavailability: a) non-specific mechanisms promoting rapid absorption of drugs and nutrients, e.g., increased blood supply to the gastrointestinal tract, decreased hydrochloric acid secretion which prevents breakdown of some drugs, increased emulsifying content of the gut, increased enzymes like gamma-glutamyl transpeptidase which participate in active and passive transport of nutrients to the intestinal cells, and b) non-specific mechanisms inhibiting enzymes participating in biotransformation of drugs, preventing their inactivation and elimination.

Se : Annamalai, A.R., Manavalan, R. (1990) Effects of 'Trikatu' and its individual components and piperine on gastroint stinal tracts: Trikatu - a bioavailable enhancer. Ind. Drugs 27(12); pp. 595-604; Johri, R.K. et al. (1992) Piperine-mediated changes in the permeability of rat intestinal epithelial cells. Bioch. Pharmacol. 43; pp. 1401-1407; Atal, C.K. et al. (1985) Biochemical basis of enhanced drug availability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. J. Pharmacol. Exp. Therap. 232; pp. 258-262; and Singh, J. et al. (1986) Piperine-mediated inhibition of glucuronidation activity in intestine: evidence that piperine lowers the endogenous UDP-glucuronic acid content. J. Pharmacol. Exp. Therap. 2236; pp.448-493.

Most drugs co-administered with piperine are probably more bioavailable as a result of both of the mechanisms, i.e., increased absorption from the gut and the slow down of biotransformation, inactivation and elimination from the system. The latter mechanism is probably the most important in sustaining the elevated blood levels of the drug, and making it more bioavailable to the tissue. Although a rapid absorption to the blood stream may account for increased blood levels of the drug, it is the inhibition of drug biotransforming enzymes with piperine that makes a drug stay in the body longer, in higher quantities, which makes it more effective.

Based on available literature data, it seems that piperine in a daily dose of at least 20 mg per person operates through inhibiting enzymes that would otherwise biotransform and speed up elimination of many drugs (Zutshi, U. et al. (1989) A process for the preparation of pharmaceutical combination with enhanced activity for treatment of tuberculosis and leprosy.

Indian Patent No. 1231/Del/89; Zutshi et al. (1984)
Influence of piperine on rifampicin blood levels in
patients of pulmonary tuberculosis. J. Assoc. Phys.
Ind. 33; pp. 223-224; Ban, C.K. et al. (1991) The effect
of piperine on the bioavailability and pharmacokinetics
of propranolol and theophylline in healthy volunteers.
European J. Clin. Pharm. 41; pp. 615-618 and Bano, G. et
al. (1978) The effect of piperine on the
pharmacokinetics of phenytoin in healthy volunteers.
Planta Medica 53; pp. 568-570).

Interestingly, the dose of piperine that
inhibits the biotransforming enzymes operates regardless
of whether it is administered concurrently with the
drug. This point can be illustrated by experiments with
theophylline and phenytoin, where 20 mg of piperine was
administered for seven days prior to the administration
of either drug [Ban, C.K. et al. (1991) The effect of
piperine on the bioavailability and pharmacokinetics of
propranolol and theophylline in healthy volunteers.
European J. Clin. Pharm. 41; pp. 615-618 and Bano, G. et
al. (1978) The effect of piperine on the
pharmacokinetics of phenytoin in healthy volunteers.
Planta Medica 53; pp. 568-570]. Since that regimen
resulted in increased blood levels of the administered
drugs, and dramatically prolonged the elimination time,
the plausible explanation is that the prior
administration of piperine inhibited drug
biotransforming enzymes. In fact, this seems to be the
only explanation for the increased bioavailability,
since piperine administered separately from the drug
could not possibly affect gastrointestinal events
leading to its rapid absorption.

Another interesting observation is that doses
of piperine below what is considered effective in
inhibiting the biotransforming enzymes, may still be

sufficient to enhance the rapid absorption of a drug from the gut. This phenomenon can be illustrated by the co-administration of piperine with the anti-hypertensive drug propranolol (Zutshi, U. et al. (1989) A process for the preparation of pharmaceutical combination with enhanced activity for treatment of tuberculosis and leprosy. Indian Patent No. 1231/Del/89).

Propranolol when administered with piperine showed a significant increase in blood levels. The maximum blood concentration of the drug increased two fold with piperine. Importantly, despite dramatically improving the bioavailability of propranolol, piperine, as used in a 3 mg dose, did not affect the elimination rate of the drug.

In an experimental design distinct from previous studies, the anti-asthmatic drug theophylline and the anti-epileptic drug phenytoin were tested (Ban, C.K. et al. (1991) The effect of piperine on the bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. European J. Clin. Pharm. 41; pp. 615-618 and Bano, G. et al. (1978) The effect of piperine on the pharmacokinetics of phenytoin in healthy volunteers. Planta Medica 53; pp. 568-570). The study was done on six healthy volunteers. The participants were pretreated with 20 mg of piperine daily for seven days before receiving 150 mg of theophylline or 300 mg of phenytoin.

The maximum concentration of theophylline was 1.5 times higher in subjects pretreated with piperine. Importantly, the elimination rate of the drug was significantly slowed down with piperine pretreatment.

Phenytoin blood concentration rose more rapidly in the group pretreated with piperine than in the group receiving the drug alone. The pretreated group attained maximum concentration of the drug in

shorter time and in significantly higher concentrations. The pretreatment with piperine resulted in significantly slower elimination of the drug.

The prior art discussed above clearly illustrates the role of piperine as a bioavailability enhancer, and the importance of its effective dose on the overall mechanism of enhanced bioavailability.

In the case of propranolol, the co-administration with only 3 mg of piperine resulted in doubling its blood levels, but without slowing down the drug elimination rate. Thus, it may be inferred that, in a small dose, piperine may not inhibit the biotransforming enzymes or affect the elimination rate of a drug. Rather, it may operate through enhancement of gastrointestinal events leading to rapid absorption mechanisms.

Summary of the Invention

The present invention is directed to preparations and methods of using such preparations to improve the bioavailability of nutritional compounds. The compositions and methods of the present invention increase gastrointestinal absorption, improve crossing over through certain biological barriers such as respiratory lining, urinary lining, blood brain barrier and skin, and systemic utilization of certain nutrients and biological compounds.

The compositions and methods of the present invention contain, as an essential ingredient, piperine. This compound may be obtained as an extract from the fruit of *piper nigrum* comprising at least 98% piperine. Alternatively, the compositions may be prepared from an extract of the fruit of *piper longum*. Piperine made synthetically may also be used in the present invention. Compositions of the present invention may also contain

extract from roots of zingiber officinale, with the active ingredients 6-gingerol and 6-shogaol. The compositions may be formulated with the extract from fruit of piper nigrum, extract from fruit of piper longum, and extract from fruits of zingiber officinale combined in any weight ratio. Preferred weight ratios include 2:2:1, 1:1:1, 2:1:1, and 1:2:1.

As a daily supplement taken with a nutrient or nutrients by an average healthy adult, piperine is effective and safe in a broad dose range. A preferred effective dose range of piperine for oral use to enhance nutrient bioavailability is 0.0004 - 0.15 mg/kg/day. The recommended dose of piperine for a healthy individual for oral use is approximately 5 mg/person/day. The recommended dose in cases of clinically diagnosed nutritional deficiencies is up to 15 mg/person/day in divided doses, i.e., 5 mg every six hours (in the morning, at noon, and in the evening). When used as a preparation for topical or parenteral use to improve crossing over through a biological barrier, the compositions of the present invention contain, as an essential ingredient, 0.00004 - 0.015 mg/kg of body weight of piperine. The nutritional materials are used in nutritionally effective amounts.

Black pepper contains approximately 5-9% piperine and is listed by the FDA as an herb which is generally recognized as safe (GRAS) for its intended use as spice, seasoning, or flavoring. The bioenhancing dose of piperine as used in the present invention is a maximum of approximately 15 mg/person/day, or no more than 20 mg/day in divided doses, which corresponds to from several thousands to up to 40,000 times less than the LD₅₀ dose of piperine, as established in various experiments on rodents.

The active ingredient of the present invention may be prepared by a novel process which produces piperine of a purity greater than 98%.

5 Brief Description of the Drawings

Figure 1 shows the mean serum β -carotene changes during piperine trial.

Figure 2 shows AUC comparisons of β -carotene 14-day bioavailability.

10 Figure 3 shows Percentage comparison of β -carotene AUC data.

Description of the Preferred Embodiments

15 The present invention is directed to preparations and methods of using such preparations to improve the bioavailability of various nutritional compounds. The compositions and methods of the present invention increase gastrointestinal absorption, improve crossing over through certain biological barriers such as respiratory lining, urinary lining, blood brain barrier and skin, and systemic utilization of certain nutrients and biological compounds.

20 The compositions of the invention also act by increasing thermogenesis. This mechanism is believed to be triggered by activation of thermoreceptors and release of catecholamines and/or direct action as beta 1, 2, 3-adrenoceptor agonist. Secretion of catecholamines can also be mediated by ATP via a P2-type purinergic receptors, and through a direct or indirect stimulation by the compositions of the invention of dopaminergic and serotonergic systems.

25 It is known that stimulation of beta-3 adrenoceptors results in increased thermogenesis, decrease in the amount of white adipose tissue without food intake being affected, increased levels of insulin

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receptors, and decreased levels of serum insulin and blood glucose. The present invention may possess anti-obesity and anti-diabetic effects, which by themselves contribute to the mechanism of thermogenesis and the increase in lean body mass.

The anti-obesity and anti-diabetic effects of the present invention can be potentiated by using the compositions of the invention in combination with vanadium, in the form of vanadium organic and inorganic salts, both synthetic and naturally occurring.

The thermogenic effect of the invention may also be mediated by an increase in the activity of thyroid peroxidase, an important enzyme in thyroid hormone synthesis, an increase in the plasma levels of triiodothyronine (T3) and thyroxine (T4) with simultaneous increase in tissue oxygen uptake and increase in thermogenesis.

The thyrogenic and thermogenic effects of the present invention can be potentiated by using the compositions of the invention in combination with L-selenomethionine and iodine supplementation.

The metabolic pathways for a nutrient and drug are different in that a nutrient sustains basic metabolism, and physiological functions of the organism, while a drug is utilized as an adjunct to basic metabolism, to restore homeostasis to the physiological functions. This distinction allows one to understand the different mechanism of bioavailability of the present invention. One mechanism, as described by the prior art, is applicable primarily to sustain therapeutic levels of a drug in the organism. The other, as discussed by the present inventors, is applicable to increase absorption of nutrients and increase their metabolic utilization.

The invention is intended primarily, but not exclusively, to increase the gastrointestinal absorption of nutrients and botanical compounds. This, in general, enhances the crossing-over of nutrients and botanical compounds through biological barriers such as, but not limited to, gastrointestinal epithelium, respiratory lining, genitourinary lining, blood brain barrier and skin.

Although not wishing to be limited to any mechanism of action, it is believed that the basic mechanism of the invention is two-fold: by affecting various active and passive transport mechanisms as described in detail below; and by causing the increase for the substrate demand due to enhanced metabolism at the cellular level. This latter mechanism is triggered when the compositions and method of the invention affect thermoregulation of the body, acting directly or indirectly through activation of thermoreceptors, which results in increased thermogenesis, or metabolic heat energy production and release. By affecting thermoreceptors, particularly in the skin and mucosa, the invention prevents thermal and non-thermal nociceptive stimuli being carried to spinal dorsal horn cells, and acts as a local and general anesthetic, by increasing threshold for the nociceptive stimuli.

The compositions of the present invention may also be used to affect thermoreceptors and prevent thermal and non-thermal noxious stimuli from being carried to the dorsal horn cells, which exerts local and general analgesic effects. In addition, piperine may potentiate the analgesic effects is believed to be caused by increasing the absorption of the analgesic with which it is administered, and additionally, providing a synergistic or additive mechanism of analgesic action.

The compositions of the present invention improve gastrointestinal absorption and systemic utilization of the nutrients and nutritional supplements. Preferred embodiments levat the maximum plasma concentration by 20-80% above the plasma concentration resulting when a regular supplement is taken alone.

The preparations of the present invention for improving gastrointestinal absorption and systemic utilization, may be made with any nutrient, biological compound, or nutritional supplement. Particularly preferred biological compounds include boswellin, curcumin, capsaicin, ashwagandha, ginkgo biloba, and aconitine.

The compositions of the present invention to improve gastrointestinal absorption and systemic utilization may also include water soluble vitamins and fat soluble vitamins. Preferred water soluble vitamins include vitamin B1, vitamin B2, niacinamide, B6, B12, folic acid, and vitamin C. The absorption of water soluble vitamins is believed to work by preventing denaturing agents present in food from altering the protein-3-tetramer hydrophilic channels which facilitate gastrointestinal absorption of water soluble vitamins. In addition, the ability of piperine to enhance gastrointestinal absorption of vitamin B12 is believed to occur by stimulating synthesis and secretion of an intrinsic factor, a glycoprotein secreted by gastric parietal cells, which facilitates transport of vitamin B12 across the cellular membrane. The preparations of the present invention used to improve gastrointestinal absorption may also contain fat soluble vitamins. Preferred fat soluble vitamins are vitamins A, vitamin D, vitamin E, and vitamin K. In addition, carotenes such as alpha-carotene, beta-carotene and transb ta-

carotene are believed to be subject to enhanced absorption due to a cholagogous mechanism which increases duodenal bile salts to emulsify fat soluble vitamins, and facilitate intracellular absorption via the mixed micelle system.

The compositions of the invention may also include amino acids, particularly the essential amino acids lysine, isoleucine, leucine, threonine, valine, tryptophan, phenylalanine, and methionine. The amino acids are believed to be subject to enhanced gastrointestinal absorption by increasing the gamma-glutamyl cycle which facilitates transmembrane transport of amino acids.

Many antibiotics decrease the absorption of certain metallic and non-metallic minerals. To offset such losses, the compositions of the present invention may include essential minerals such as iodine, calcium, iron, zinc, copper, magnesium and potassium. Other metals such as vanadium, chromium, selenium and manganese may also be included in compositions of the present invention. It appears that the compositions of the present invention facilitate gastrointestinal absorption of these metallic compounds mainly by enhancing the active transport of these compounds across the membrane. In addition, the compositions of the present invention may prevent gastrointestinal absorption of certain dangerous heavy metals such as lead, mercury, and cadmium, and prevent systemic interference of the heavy metals with enzymatic functions. The compositions of the invention can form insoluble salts with mercury chloride, lead chloride and cadmium chloride and also protect sulfhydryl groups of enzymes from reacting with heavy metals.

The compositions of the present invention may also include antioxidants. Preferred antioxidants

includ vitamin A, vitamin C, vitamin E, alpha-carot n ,
transbeta-carotene, betacryptoxanthin, lycop ne,
lutein/zeaxanthin, pin bark bioflavonals complex,
germanium, sel nium, and zinc. The enhancement of
antioxidant activity is b lieved to occur through
enhanced systemic availability of antioxidant compounds
through inhibition of lipid peroxidation and free
radical formation. Preferred compositions of the
present invention may include a variety of any of the
above ingredients, which are particularly needed in a
particular population.

Many of the nutrients, biological compounds
and nutritional supplements which may be included in the
compositions of the present invention are available
commercially. Particularly, vitamin, mineral, amino
acid and antioxidants are available commercially. The
herbal compounds are generally used in powder form which
is a dried ethanol extract of a particular plant. For
example, Boswellic acid is from an ethanol extract of
Boswellia serrata roots. Ginsenosides are from an
ethanol extract of Ginseng roots. Withanalooids are from
an ethanol extract of Whitania somnifera plant. Gingko
flavinoids are from an ethanol extract of Gingko biloba
plant. Curcuminoids are from ethanol extract of Cucuma
longa plant. Pycnogenol is from an ethanol extract of
Pinus pinaseter bark. Proanthocyanidins are from an
ethanol extract of pine bark. Some of the herbal
compounds are also available commercially from a variety
of sources. The piperine in the invention may be
produced by the new method of isolation of piperine
shown in Example 14, below. The compound obtained in
this manner has the trademark name of Bioperine®.
Alternatively, piperine may be provided by the prior art
methods or made synthetically.

The following examples are not intended to be limiting in any way, but demonstrate some of the preferred embodiments of the present invention.

EXAMPLES

NUTRITIONAL FORMULATIONS

Vitamin A tablets/capsules

Example 1)

Formulation: Vitamin A tablets/capsules/softgels

Vitamin A (Palmitate)	10,000 IU
Piperine	4 mg

Example 2)

Vitamins A & D tablets/capsules/softgels

Vitamin A (Palmitate)	10,000 IU
Vitamin D (Calciferol)	400 IU
Piperine	4 mg

Example 3)

Betacarotene capsules/softgels

Betacarotene	15 mg
Piperine	4 mg

Example 4)

Curcumin Capsules

Curcumin (min. 95% of curcuminoids)	500 mg
Piperine	5 mg

Example 5)

Boswellin Capsules

Boswellia Serrata Extract	320 mg
(65% Boswellic acid min.)	
Piperine	3 mg

Example 6)

Herbal capsules/tablets

Formula A

	Valerian root	100 mg
	Chamomile (flower)	100 mg
	Passion Flow r	25 mg
5	Ginseng root powd r	50 mg
	Skullcap	25 mg
	Nettle leaves	25 mg
	Piperine	3 mg

10

Formula B

	Buchu leaves	100 mg
	Uva Ursi leaves	25 mg
	Celery seed	25 mg
	Juniper berries	50 mg
15	Parsley leaves	50 mg
	Corn silk	50 mg
	Piperine	4 mg

20

Formula C

	Echinacea root	100 mg
	Astragalus root	100 mg
	Barley leaves	50 mg
	Schizandra berries	100 mg
25	Shiitake Mushroom	50 mg
	Piperine	5 mg

Formula D

	Ginseng extract	500 mg
	(5% Ginsenosides)	
30	Piperine	5 mg

Formula E

	Ginseng extract	250 mg
35	Ashwagandha extract (1% withanoloids)	250 mg
	Piperine	5 mg

Formula F

	Ginkgo Biloba extract	
	(24% ginkoflavinoids)	240 mg
40	Piperine	3 mg

Formula G

	Boswellia Serrata extract	320 mg
45	Curcumin	200 mg
	Piperine	5 mg

Formula H

	Boswellia Serrata extract	320 mg
50	Capsaicin	3 mg
	Piperin	4 mg

Exempl 7)

Anti-oxidant tabl ts/capsules

5	<u>Formula A</u>	
	Vitamin C	250 mg
	Vitamin E	100 IU
	Vitamin A (Beta Carotene)	10,000 IU
10	Selenium	50 µg
	(from L-Selenomethionine)	
	Chromium	50 µg
	(Chromium Picolinate)	
	Piperine	4 mg
15	<u>Formula B</u>	
	Pycnogenol	30 mg
	Piperine	3 mg
20	<u>Formula C</u>	
	Pine bark extract	15 mg
	Curcumin	15 mg
	Piperine	3 mg
25	<u>Formula D (softgel)</u>	
	Coenzyme Q ₁₀	15 mg
	Piperine	3 mg
30	<u>Formula E - Anti-oxidant beverage drink</u>	
	Vitamin C	200 mg
	Beta carotene	15 mg
	Vitamin E	100 IU
	Zinc (monomethionine)	15 mg
	Selenium (L-Selenomethionine)	50 µg
35	Citrus bioflavanoid complex	50 mg
	Quercetin	25 mg
	Rutin	25 mg
	Hesperidin (Citrus)	20 mg
	Pycnogenol	5 mg
40	Piperine	2.5 mg

Example 8)

45 Amino Acid Formulation

	L-Taurine	200 mg
	L-Carnitine	100 mg
50	Piperine	2.5 mg

Example 9)

Vitamin B C mplex

Pantoth nic Acid (Vitamin B5)	200 mg
Niacinamide (Vitamin B5)	125 mg
Pyridoxine HCL (Vitamin B6)	75 mg
Thiamine (Vitamin B1)	60 mg
Riboflavin (Vitamin B2)	25 mg
Para-aminobenzoic acid (PABA)	25 mg
Folic acid	400 µg
Cobalamin (Vitamin B12)	200 µg
Biotin	100 µg
Piperine	4 mg

Example 10)

Multi Vitamin

Vitamin A	5,000 IU
Vitamin B1	1.5 mg
Vitamin B2	1.7 mg
Vitamin B6	2.0 mg
Niacinamide	20 mg
Vitamin E	30 IU
Vitamin B12	6 mg
Pantothenic Acid	10 µg
Vitamin D	400 IU
Vitamin C	100 mg
Folic Acid	400 µg
Biotin	30 µg
Calcium	200 mg
Magnesium	400 mg
Iron	18 mg
Iodine (Kelp)	150 µg
Copper	2 mg
Manganese	2.5 mg
Potassium	40 mg
Chromium	25 mg
Selenium	25 mg
Vitamin K1	25 mg
Piperine	5 mg

Example 11)

Hydroxycitric Acid

Citrin®	500 mg
(Calcium salt of hydroxycitric acid)	
Piperine	5 mg

The above formulations and ingredients are examples, and are not intended to limit the invention in any way.

Example 12

Bioavailability of beta-carotene

The following is an example of the utilization of piperine in nutrient absorption. Piperine known under the trademark Bioperine® consisting of 98% piperine has been evaluated in a nutrient bioavailability experiment. The objective of the study was to compare the beta-carotene, in human volunteers receiving the formula with and without Bioperine®. Twelve healthy male volunteers, non-smokers, abstaining from alcohol, not taking nutritional supplements or prescription drugs during the period of cross-over study, received beta-carotene supplementation with and without Bioperine® for a period of 14 days.

TABLE 1. Formulations of beta-carotene alone and with Bioperine® used in the 14 day double-blind, cross-over study

The following formulations of beta-carotene were tested:

	Formulation A	Formulation B
Beta-carotene	15 mg	15 mg
Methylcellulose	480 mg	480 mg
Bioperine®	None	5 mg

Results of this study demonstrate that after 14 days of supplementation, volunteers from the control group, receiving formulation A, were found to have significantly smaller increase in blood levels of beta-carotene than those from the group receiving formulation B with Bioperine®. The group receiving Bioperine® had

almost a two fold increase in blood beta-carotene levels as compared to the group receiving beta-carotenes only.

TABLE 2. Blood levels of beta-carotene after 14 days supplementation of beta-carotene alone or beta-carotene with Bioperine®

Pharmacokinetic parameters for beta-carotene

	Formulation A	Formulation B
Maximum plasma conc. ug/dl AUC*	47.2 + 6.4	65.8 + 9.7**
ug/dl/day	272.0 + 47.6	435.2 + 74.2**

* Area under the plasma beta-carotene during 14 day supplementation

** highly significant ($p < 0.001$).

The probable mechanism of piperine's effect on the bioavailability of beta-carotene is its effect on gastrointestinal events that lead to increased absorption of this nutrient and most probably other nutrients as well.

A dose of 5 mg of piperine, and up to 15 mg, would most likely not interfere with the metabolism of a majority of drugs as previously discussed. In fact, this dose as used with beta-carotene does not affect the metabolic pathways of this nutrient in the body, as measured by the blood levels of retinol which remained unchanged throughout the experiment (see below).

Retinol, or vitamin A, is a product of metabolic conversion of beta-carotene, and its blood levels would likely be affected by enzymatic inhibition/stimulation with piperine. It is an important finding that piperine does not elevate the conversion of beta-carotene to vitamin A, since toxic effects due to an overdose of vitamin A are well known. The benefit of increased

blood levels of beta-carotene without danger of vitamin A toxicity translates into the safe and effective enhancement of anti-oxidant protection provided by beta-carotene.

TABLE 3. Values of Vitamin A (retinol) before treatment and after 14 days supplementation with Bioperine®

Formulations	Before treatment (ug/dL)	Day 14 (ug/dL)
A	66.0 + 11.5	65.1 + 10.2*
B	65.2 + 11.9	65.0 + 10.6*

*difference not statistically significant

Additional evidence from the experiment with 14 day supplementation of beta-carotene to 12 healthy volunteers show that piperine does not affect absorption, metabolism and elimination of nutrients that are not supplemented, but otherwise consumed with a daily food. For example, the blood levels of water soluble vitamin C and lipid soluble vitamin E, which were not supplemented, were not affected as assessed before the study and after completion of 14 day supplementation of beta-carotene with piperine.

TABLE 4. Comparison of vitamin C blood levels before and after 14 day supplementation with beta-carotene or beta-carotene with Bioperine®

Formulations	Before treatment (ug/dl)	Day 14 (ug/dl)
A	0.62 + 0.13	0.64 + 0.10*
B	0.66 + 0.12	0.65 + 0.11*

*difference not statistically significant

TABLE 5. Comparison of vitamin E blood levels before and after 14 day supplementation with beta-carotene and Bioperin

Formulations	Before treatment (ug/dl)	Day 14 (ug/dl)
A	0.80 + 0.18	0.84 + 0.15*
B	0.83 + 0.16	0.84 + 0.16*

*difference not statistically significant

The apparent lack of any inhibitory effect from piperine on human metabolism at low doses is an important observation. This is particularly so because many of those who may require piperine co-administered with a nutrient like certain vitamins, may also be on a drug regimen which can not be altered. Based on the available literature, a dose of piperine below 20 mg per day per person should not affect the metabolism of most if not all xenobiotics such as the drugs discussed above.

Example 13

Toxicity

Black pepper, which contains approximately 5 - 9% of piperine, is listed by the FDA as an herb generally recognized as safe (GRAS) for its intended use as a spice, seasoning and flavoring (21 CFR 100.0, 182.10, 182.20). Based on black pepper imports, it is estimated that the average consumption of black pepper in the United States is about 359 mg/person/day*. This amount of crude pepper translates to 18.0 - 32.3 mg of piperine/person/day [Table 6].

Table 6. Estimated average human consumption of black pepper/piperine*

	mg/p rson	mg/kg**
Black pepper daily	359	6.0
Piperine daily	18.0 - 32.3	0.3 - 0.54

* Data based on doctoral thesis by Shore Scott Kindell, Drexel University, 1984.

** Average weight of a person estimated at 60kg.

According to other sources daily human consumption of black pepper constitutes 0.02% of the diet, which corresponds to 2 mg of powdered pepper/kg/day (Bhat, G.B. Chandrasekhara, N. (1986), "Lack of adverse effect of piperine, pepper or pepper oleoresin on weanling rats", J. Food Safety, 7; pp. 215-223). Based on this assumption, black pepper, and its components oleoresin containing 40% piperine, and pure piperine, were fed to rats at doses calculated as 5 to 20 times the average daily intake for humans. This particular diet with pepper and its components did not affect food intake, growth pattern of fed animals, the organ weights, and produced no clinical symptoms. Comparison of the blood chemistry tests results of the treated and untreated animals showed no alterations in RBC, WBC, the differential count, levels of hemoglobin, total serum proteins, albumins, globulins, glucose, cholesterol and levels of serum aminotransferases and phosphatases.

Acute, subacute and chronic toxicity studies of piperine in laboratory animals indicate that piperine used in broad range of doses, does not cause any abnormality in the general growth pattern, body to organ weight ratio, clinical symptomatology and blood chemistry data (Johri, R.K., Zutshi, U. (1992), "An Ayurvedic formulation 'Trikatu' and its constituents",

Journal of Ethnopharmacology, 37; pp. 85-91). The dose of piperine considered as bioenhancing absorption of nutrients is considered 0.0004 - 0.15 mg of piperine/kg body weight. That is many thousand times less than the LD₅₀ dose (dose toxic to 50% animal tested) of piperine established in mice and rats [Table 7]. The LD₅₀ data indicate a relatively high therapeutic index for piperine, which means high degree of safety in nutritional use of piperine.

Table 7. Piperine LD₅₀ dose established in rodents (Johri, R.K., Zutshi, U. (1992), "An Ayurvedic formulation 'Trikatu' and its constituents", Journal of Ethnopharmacology, 37; pp. 85-91 and Plyachaturawat, P. et al. (1983), "Acute and sub-acute toxicity of piperine in mice, rats and hamsters", Toxicology Letters, 16; pp. 351-359).

Experimental animal	LD ₅₀ mg/kg body weight
Mice	1,638.6
Mice	330.0
Rat weanling	585.0
Rat	514.0
Rat	800.0

Piper species have been traditionally used to induce abortion. The reported antifertility property of piperine was investigated in laboratory animals (Plyachaturawat, P. et al. (1982), "Post-coital antifertility effect of piperine", Contraception, 26; pp. 625-633 and Kholkute, S.E. et al. (1979), "Antifertility activity of the fruits of piper longum in

female rats", Indian J. Exp. Biol., 17; 289-290).

Piperine inhibited significantly pregnancy in mice when given by either intraperitoneal or oral route of administration at a dose of 12.5 mg/kg body weight, twice a day. Piperine was effective at both pre- and post-implantation periods. However, when used in a dose of 5 mg/kg body weight/day, which is approximately 70 times more than the bioenhancing dose, piperine had no anti-fertility activity in mice. The dose of piperine that significantly inhibited pregnancy in mice did not interfere with the estrogen cycle, did not show uterotrophic effect, and did not produce clinically noticeable toxicity. The mechanism of anti-fertility action of piperine is not known at present, but it does not operate through a hormonal mechanism or uterotonic activity.

Black pepper extracts have been shown to possess tumor inhibitory activity (Loder, J.W. et al. (1969), "Tumor inhibitory plants", Aust J. Chem., 22; pp.1531-1538), but one report points to a possibility of carcinogenic effect of black pepper (Concon, J.M. et al (1979), "Black pepper: Evidence of carcinogenicity", Nutrition and Cancer, 1; pp. 22-26). In this study an ethanol extract of black pepper at a dose calculated as 400 to 700 times higher than the pharmacologically effective dose of piperine, has been applied topically to mice for three months. This treatment resulted in the significant increase in tumor occurrence in mice. The authors of this report discuss several chemicals that are known components in black pepper extracts as possible culprits. Safarole, tanins and terpenoids like d-limonene, l-pinene, linalool and phellandrene are specifically mentioned as potential carcinogens, cocarcinogens, or tumor promoters. Piperine has not been implicated directly by the report as a possible

tumorigenic compound, but because it contains the methylendioxybenzene structure in common with safarole, chavicine, piperittine and myristicine may be problematic. However, experiments dealing with piperine and safarole metabolism have shown that despite chemical likeness, piperine and safarole are metabolized differently, giving rise to different types of products (Ionnoids, C. et al. (1981), "Safrole: Its metabolism, carcinogenicity and interactions with cytochrome P-450", Food and Cosmetics Toxicology, 19; pp. 657-666 and Bhat, B.G., Chandrasekhara, N. (1987), "Metabolic Disposition of Piperine in the Rat", Toxicology, 44: pp. 99-106). Piperine and safarole interact differently with rat liver microsomes, and those differences may be attributed to the structural dissimilarities in the side chain of these two compounds (Wrba, H. et al. (1992) "Carcinogenicity testing of some constituents of black pepper (*Piper nigrum*), 44(2); pp. 61-65.) For example, the cytochrome P-450 content of hepatic microsomes decreased by approximately 20% due to treatment of rats with piperine, whereas safarole pretreatment increased the cytochrome P-450 content by about 50%.

In a separate experiment, preweaned mice injected with safarole, tannic acid or methylcholantrene, a reference carcinogen, developed tumors. Safarole and tannic acid have been shown to be weak carcinogens when compared with methylcholantrene. Feeding of d-limonene to the mice which were injected with any of the above three substances reduced their carcinogenic activity. Feeding piperine to the mice receiving the three compounds did not modify their carcinogenic activity (Wrba, H. et al. (1992) "Carcinogenicity testing of some constituents of black pepper (*Piper nigrum*), 44(2); pp. 61-65).

Owing to its interaction with xenobiotic-metabolizing enzymes, piperine may potentially inhibit detoxification of some toxic compounds. To investigate this possibility the in vitro influence of piperine on various enzymes that are closely related to the metabolism and detoxification system of a toxic compound such as benzo(a)pyrene has been studied (Chu, C.Y. et al. (1994), "Modulatory effect of piperine on benzo(a)pyrene-induced cytotoxicity and DNA adduct formation in V-79 lung fibroblasts", Food and Chem. Toxicol., 32 (4); pp. 373-377). This study showed that piperine significantly enhanced benzo(a)pyrene cytotoxicity in vitro. The results of this study are contradicted, however, by another report indicating that piperine can in fact inhibit benzo(a)pyrene activation, by lowering benzo(a)pyrene-oxide production, thus decreasing benzo(a)pyrene cytotoxicity (Atal, C.K. et al. (1985), "Biochemical basis of enhanced drug bioavailability by piperine: evidence that piperine is a potent inhibitor of drug metabolism", J. Pharmacol. Exp. Therap., 232; pp. 258-262).

Alteration of hepatic mixed function oxidases may potentially lead to hepatotoxic effect. Piperine was evaluated for potential hepatotoxicity in rats (Dalvi, R.R., Dalvi, P.S. (1991) "Differences in the effect of piperine and piperonyl butoxide on hepatic drug metabolizing enzymes in rats", Drug Metab. Drug Interact., 9(1); pp. 23-30). Piperine was administered to the animals intragastrically in doses 100 mg and 800 mg/kg, and intra-peritoneally in doses 10 mg/kg and 100 mg/kg. None of the treatment regimens altered significantly the following liver enzymes indicative of liver damage: serum sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and isocitrate dehydrogenase

(ICD).

In a separate study piperine was found to have a hepatoprotective action in mice treated with potent hepatotoxins tetra-hydroperoxid and carbon tetrachloride (Koul, I.B., Kapil, A. (1993), "Evaluation of the liver protective potential of piperine, an active principle of black and long peppers", Planta Med., 59(5); pp. 413-417). This protective effect, demonstrated by both in vivo and in vitro experiments, was explained by piperine mediated decrease in lipid peroxidation, and reduction in hepatocellular damage as measured by reduced enzymatic leakage of glutamate pyruvate transaminase and alkaline phosphatase. Piperine also prevented depletion of reduced glutathione and total thiols in the liver. The reduced glutathione is one of the most important biomolecules in protection against chemically induced cytotoxicity, and can eliminate toxic compounds by conjugation.

The effect of piperine on lipid peroxidation and liver enzymes was confirmed in other studies, which show piperine inhibiting lipid peroxide formation and leveling off increased levels of acid phosphatase in rats injected with carageenin - a compound which is known to stimulate liver peroxide formation (Dhuley, J.N. et al. (1993), "Inhibition of lipid peroxidation by piperine during experimental inflammation in rats", Ind. J. Exp. Biol., 31; pp. 443-445). Liver peroxide output is increased as a result of damage of lysosomes probably elicited indirectly by inflammatory action of carageenin, with subsequent increased levels of liver lipid peroxidation and acid phosphatase.

The nitrosation reaction of piperine is of concern, as endogenous nitrosation could take place in the human stomach from ingested piperine and nitrites (Wakabayashi, K. et al. (1989), "Mutagens and

carcinogens produced by the reaction of environmental aromatic compounds with nitrite", Canc. Surv., 8(2); pp. 385-399). This combination may lead to potentially mutagenic products. Nitrites can be ingested by consuming cured foods, such as meat and, in particular, bacon. Nonetheless, research data indicate a remote possibility of any potential nitrosation reaction of piperine, for the following reasons:

* average consumption of pepper for culinary purposes and proposed dose of piperine for pharmacological effects are well below doses of piperine required for the significant nitrosation reaction to occur;

* a number of food constituents, such as ascorbic acid, tocopherols, plant phenolics and flavanoids exert protective effect against nitro derivatives formation. Thus, nitro compounds formation with piperine, if any, would be a multifactorial and competitive event;

* recent study indicates that due to awareness of deleterious health effects of the volatile nitrosamines, a continuously lowering trend in the levels of volatile nitrosamines in all types of food, with exception of fried bacon, is being noticed.

The doses of piperine, recommended for bioavailability enhancement are relatively low when compared to the toxic doses, and translate to a dose of pure piperine in a range of 2.5 to 5 mg per dose. That dose equals to an average daily dose of 0.04 - 0.25* mg of piperine/kg body weight [Table 8].

Table 8. Average human consumption of piperine as a bioenhancing compound

	mg/person	mg/kg**
Bioperine daily	2.5 - 15*	0.04 - 0.25

Bioperin ® monthly	75 - 450	1.25 - 7.5
Bioperine yearly	900 - 5,400	15 - 900

* Daily dose calculated based on lowest and highest estimated dose of Bioperin used as bioenhancing supplement 1 - 3 times daily.

** Average weight of a person estimated at 60 kg.

Example 14

A preparation obtained in a unique manufacturing process.

Commercially available Black pepper oleoresin or Long pepper oleoresin is used as the source of piperine. Ground up Black pepper or Long pepper can also be used.

To a mixture of butanol and hexane (35 liters), 35 kg Black pepper oleoresin is added and heated to 40°C. The mixture is then cooled and filtered.

The precipitate is washed with Butanol/hexane mixture to obtain crude piperine.

The crude piperine is dissolved in methanol at 60°C and treated with alumina and charcoal by stirring. It is then filtered and concentrated under vacuum to obtain a powder.

Bioperine®

Material thus prepared has the following specifications: Color: Pale yellow crystalline powder

Melting range: 128 degrees - 131 degrees Celsius

Assay: min. 98% pure piperine (by HPLC)

CLAIMS

We claim:

1. A composition suitable for improving gastrointestinal absorption and systemic utilization of nutritional materials, substantially increase in nutrient induced thermogenesis, and increase in lean body mass, comprising at least one nutritional material in a nutritionally effective amount and a bioavailability enhancing effective amount of a bioavailability enhancer comprising at least 98% of the alkaloid piperine.
2. The composition of claim 1 wherein the piperine is obtained from an extract of fruits of the family Piperaceae.
3. The composition of claim 1 wherein the piperine is extracted from the fruit of *piper nigrum* or black pepper.
4. The composition of claim 1 wherein the piperine is extracted from the fruit of *piper longum*.
5. The composition of claim 1, which contains additionally an extract from the root *zingiber officinale*.
6. The composition of claim 1 wherein the nutritional materials comprise one or more members selected from the group consisting of herbal extracts, water soluble vitamins, fat soluble vitamins, amino acids, minerals, anti-oxidants, and combinations containing at least two of the nutritional materials.
7. The composition of claim 6 wherein the herbal extracts comprise one or more members selected from the

group consisting of curcumin, boswellin, ashwagandha, ginkgo biloba, capsaicin, and aconitine.

8. The composition of claim 6 wherein the water soluble vitamins comprise one or more members selected from the group consisting of B1, B2, niacinamide, B6, B12, folic acid, and vitamin C.

9. The composition of claim 6 wherein the antioxidants comprise one or more members selected from the group consisting of vitamin A, vitamin C, vitamin E, alpha-carotene, transbeta-carotene, betacryptoxanthin, lycopene, lutein/zeaxanthin, pine bark bioflavonals complex, germanium, selenium, and zinc.

10. The composition of claim 6 wherein the amino acids comprise one or more members selected from the group consisting of lysine, isoleucine, leucine, threonine, valine, tryptophan, phenylalanine, methionine, and L-selenomethionine.

11. The composition of claim 6 wherein the minerals comprise one or more members selected from the group consisting of calcium, iron, zinc, vanadium, selenium, chromium, iodine, potassium, manganese, copper, and magnesium.

12. The composition of claim 1 which contains an extract from the fruit of piper nigrum, an extract from the fruit of piper longum, and an extract from the root of zingiber officinale.

13. The composition of claim 1, suitable for oral administration, in a unit dose form of 0.0004-0.15 mg/kg of body weight.

14. The composition of claim 1, suitable for topical or parent ral use, containing the unit dose of 0.00004-0.015 mg/kg of body weight.

15. A method f r improving gastrointestinal absorption and systemic utilization of nutritional materials subsequent increase in nutrient induced thermogenesis, and increase in lean body mass; said method comprising administering a composition containing at least one nutritional material, a bioavailability enhancing effective amount of a bioavailability enhancer comprising at least 98% alkaloid piperine, to a subject in need of such treatment.

16. The method of claim 15 wherein the piperine is obtained from an extract from the fruits of the family Piperaceae.

17. The method of claim 16 wherein the piperine is extracted from the fruit of piper nigrum or black pepper.

18. The method of claim 16 wherein the piperin is extracted from the fruit of piper longum.

19. The method of claim 15, which contains additionally an extract from the root zingiber officinale.

20. The method of claim 15 wherein the nutritional materials comprise one or more members selected from the group consisting of herbal extracts, water soluble vitamins, fat soluble vitamins, amino acids, minerals, anti-oxidants, and combinations containing two or m r of th above nutritional materials.

21. The method of claim 20 wherein the herbal extracts comprise one or more members selected from the group consisting of curcumin, boswellin, ashwagandha, ginkgo biloba, capsaicin, and aconitin .

22. The method of claim 20 wherein the water soluble vitamins comprise one or more members selected from the group consisting of B1, B2, niacinamide, B6, B12, folic acid, and vitamin C.

23. The method of claim 20 wherein the antioxidants comprise one or more members selected from the group consisting of vitamin A, vitamin C, vitamin E, alpha-carotene, transbeta-carotene, betacryptoxanthin, lycopene, lutein/zeaxanthin, pine bark bioflavonals complex, germanium, selenium, and zinc.

24. The method of claim 20 wherein the amino acids comprise one or more members selected from the group consisting of lysine, isoleucine, leucine, threonine, valine, tryptophan, phenylalanine, methionine, and L-selenomethionine.

25. The method of claim 20 wherein the minerals comprise one or more members selected from the group consisting of calcium, iron, zinc, vanadium, selenium, chromium, iodine, potassium, manganese, copper, and magnesium.

26. The method of claim 15 which contains an extract from the fruit of piper nigrum, extract from the fruit of piper lungum, and extract from the root of zingiber officinale.

27. The method of claim 15 wherein the composition is administered orally.

28. The method of claim 15 wherein the composition is administered topically or parenterally.

29. The method of claim 15 wherein the composition is administered topically.

30. The method of claim 16 wherein the composition is administered parenterally.

31. The method of claim 16 wherein the animal is a nonhuman animal.

32. The method of claim 16 wherein the animal is a mammal.

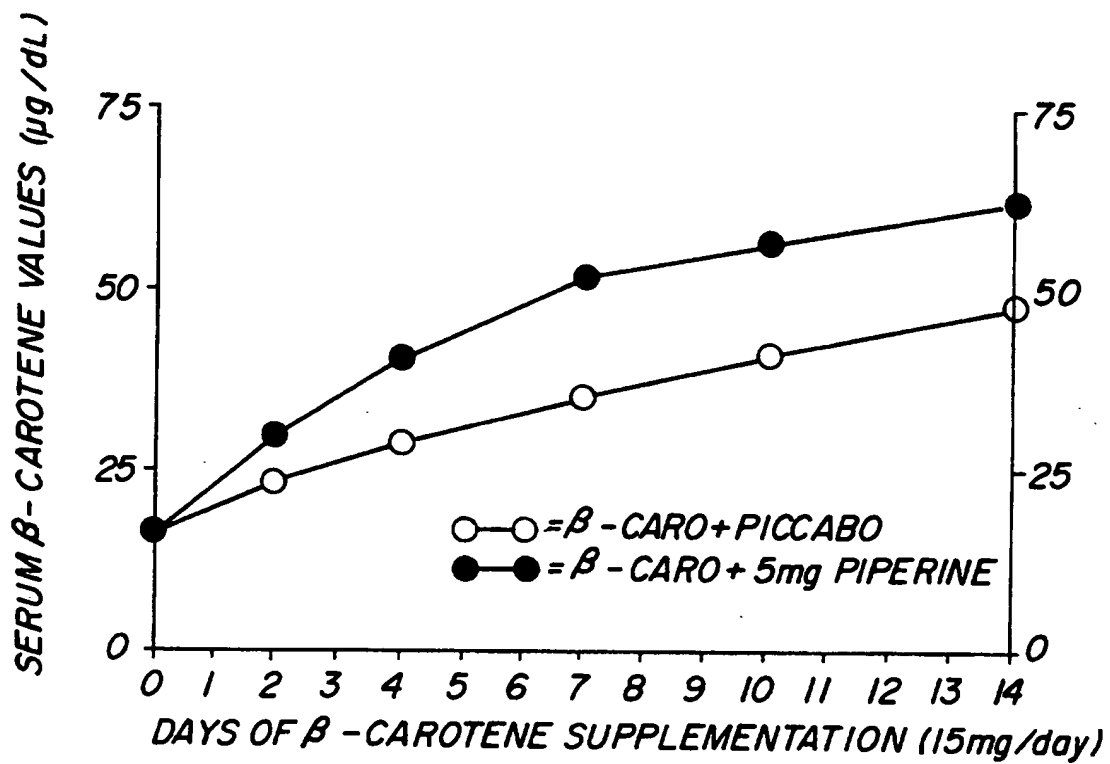
33. A method for the purification of piperine to greater than 98% comprising

- a) providing a source of piperine;
- b) adding the material of step a) to butanol and hexane;
- c) heat to 40° Celsius;
- d) cooling and filtering the material of step c);
- e) washing the precipitate with butanol/hexane mixture to obtain crude piperine;
- f) dissolving the material of step e) in methanol at 60° C;
- g) adding alumina and charcoal by stirring to the material of step f) and filtering; and
- h) concentrating the filter under vacuum.

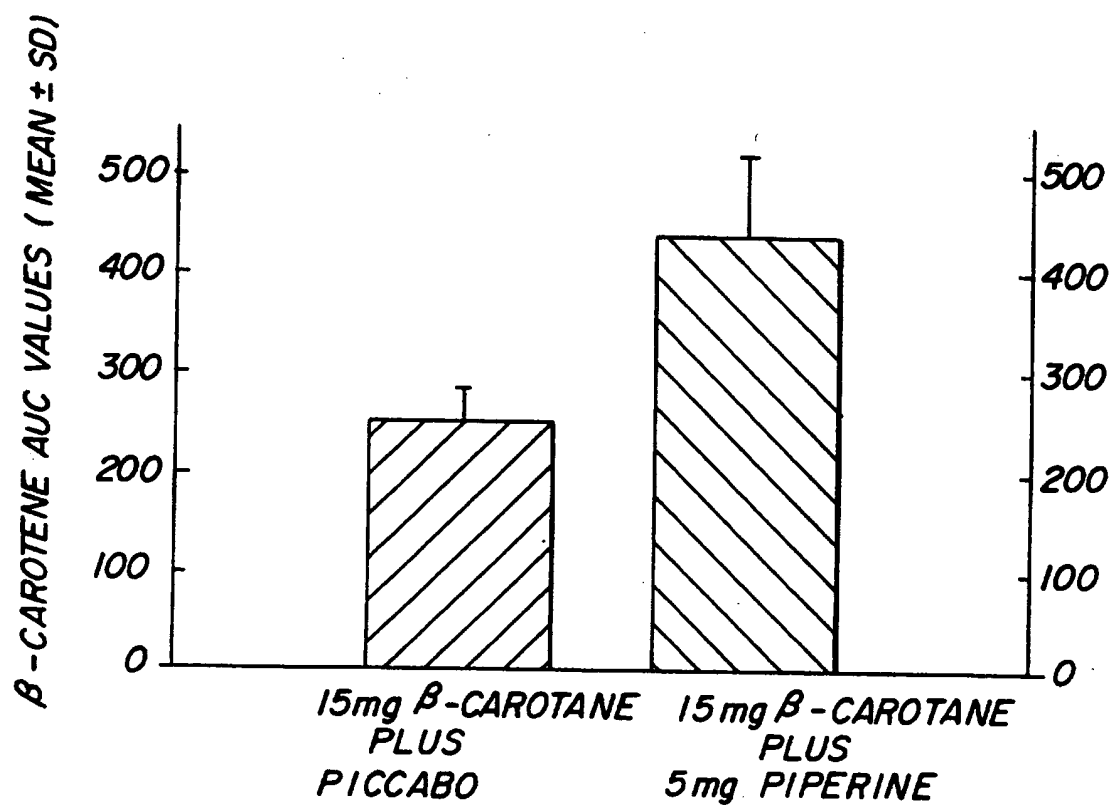
34. The method of claim 33 wherein the source of piperine is oleoresin from black pepper or long pepper.

35. The method of claim 33 wherein the source of piperine is ground black pepper or long pepper.

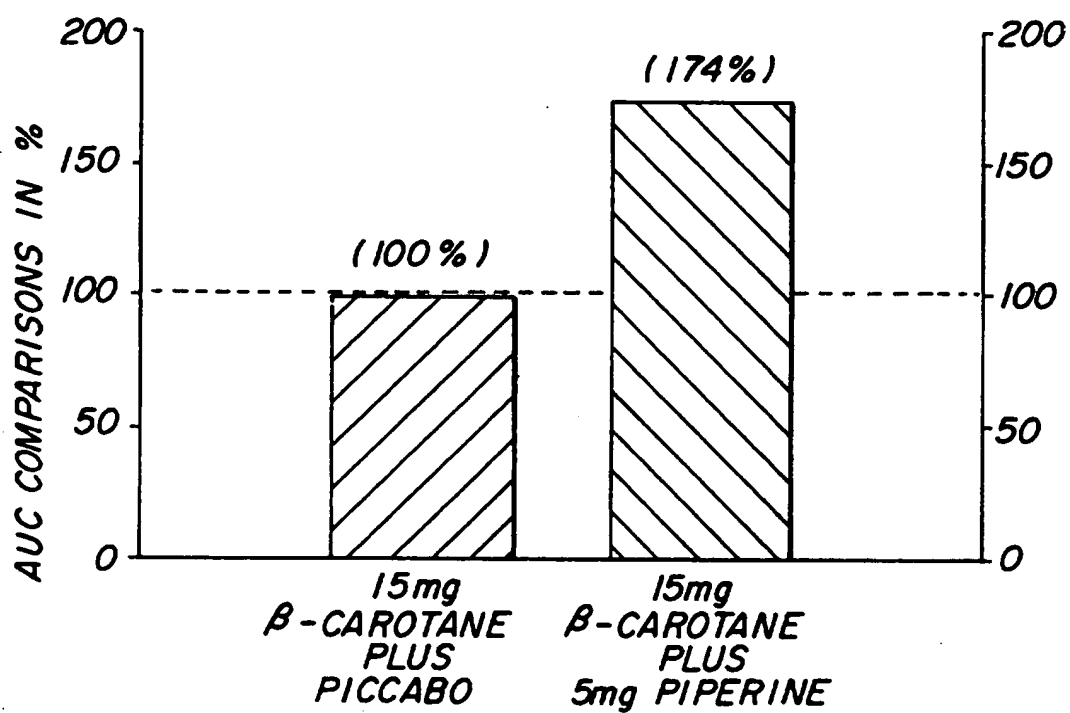
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*Fig. 1*

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*Fig. 2*

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*Fig. 3*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12758**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 35/78; B01D 11/04, 24/46, 35/18

US CL :424/195.1; 210/634, 768, 772, 774, 806

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/195.1; 210/634, 768, 772, 774, 806

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,284,657 (STANTON) 18 August 1981, see entire document.	1-32
A	Flavour Fragrance Journal, Vol. 3(2), issued 1988, Wood et al., "Piperine determination in pepper and its oleoresins-a reversed-phase high performance liquid chromatographic method", 55-64, see Chemical Abstract No. 110:6454.	33-35



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
*A	document defining the general state of the art which is not considered to be of particular relevance		
*E	earlier document published on or after the international filing date	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O	document referring to an oral disclosure, use, exhibition or other means		
*P	document published prior to the international filing date but later than the priority date claimed	*A	document member of the same patent family

Date of the actual completion of the international search

02 JANUARY 1996

Date of mailing of the international search report

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